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Synthetic biology platform technologies for antimicrobial applications☆

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ABSTRACT

The growing prevalence of antibiotic resistance calls for new approaches in the development of antimicrobial therapeutics. Likewise, improved diagnostic measures are essential in guiding the application of targeted therapies and preventing the evolution of therapeutic resistance. Discovery platforms are also needed to form new treatment strategies and identify novel antimicrobial agents. By applying engineering principles to molecular biology, synthetic biologists have developed platforms that improve upon, supplement, and will perhaps supplant traditional broad-spectrum antibiotics. Efforts in engineering bacteriophages and synthetic probiotics demonstrate targeted antimicrobial approaches that can be fine-tuned using synthetic biology-derived principles. Further, the development of paper-based, cell-free expression systems holds promise in promoting the clinical translation of molecular biology tools for diagnostic purposes. In this review, we highlight emerging synthetic biology platform technologies that are geared toward the generation of new antimicrobial therapies, diagnostics, and discovery channels.

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☆ Synthetic biology: Innovative approaches for therapy

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1. Introduction

The growing prevalence of antibiotic-resistant pathogens calls for the development of a new generation of antimicrobial therapies, diagnostics, and discovery platforms. The Centers for Disease Control and Prevention (CDC) reports that the rise of antibiotic resistance has become a public health crisis, leading to over 2 million infections and 23,000 deaths per year in the United States alone [1]. Limited diagnostic capabilities leave healthcare providers unable to precisely diagnose clinical infections and administer effective treatments in a timely manner. Furthermore, the use of broad-spectrum antibiotics clears out microbial niches and enables microbiome colonization by opportunistic pathogens [2]. This threat has motivated efforts to reinvigorate antibiotic research and streamline funding and approval processes for new therapies [3]. However, the rapid transmission of antibiotic resistance challenges our present ability to develop additional small-molecule therapeutics, broad spectrum or otherwise [4]. Amidst this antibiotic crisis, new research approaches are needed to progress the discovery of novel antimicrobial treatments.

Synthetic biology is well positioned to address the challenges in developing next-generation antimicrobial agents. By studying the design principles underlying native gene networks, synthetic biologists have developed tools that enable the rational engineering of biological systems [5,6]. Early efforts focused on the development of model transcriptional networks to recapitulate and understand native gene regulation [7–10]. These works advanced our ability to engineer complex behavior such as memory encryption and oscillatory gene expression, and catalyzed advancements in the rapid design and implementation of synthetic gene networks [11–20]. The field has since moved towards repurposing natural biological processes for tunable and targetable synthetic gene regulation [21–25]. The innate biochemistry of microorganisms has been harnessed in the biosynthesis of organic compounds, such as the antimalarial drug artemisinin [26] and various opioids [27]. Strides have also been made in engineering genetic networks for direct clinical applications such as customized cancer treatments and nonconventional cell therapies [28,29]. The extensive knowledge base that has emerged from synthetic biology and its union with other scientific fields, combined with the pressing need for next-generation antimicrobials, has led to the creation of new methods to combat

pathogen resistance. In this review, we highlight synthetic biology platform technologies that can be utilized to produce novel antimicrobial therapeutics and diagnostics.

2. Therapeutics

Current broad-spectrum antibiotic treatments often result in the elimination of both pathogenic and commensal microorganisms. This clearing out of microbial niches leads to iatrogenic infections such as *Clostridium difficile* (*C. difficile*) and contributes to the increasing prevalence of antibiotic-resistant microbes [30,31]. Synthetic biology approaches have demonstrated potential in the development of targeted therapies that improve on existing treatment schemes by narrowing the antimicrobial target spectrum and providing spatiotemporal control over the delivery of therapeutic agents.

2.1. Bacteriophage-based therapeutics

One area of research that holds promise towards such applications is that of bacteriophage-based therapeutics (Fig. 1A). Bacteriophages (phages) are bacteria-specific viruses that insert their genetic information into a host microbe upon infection, taking over essential cellular functions and in some cases inducing microbial lysis [32]. Phages naturally discriminate between microbial species within mixed populations and bear great specificity toward their bacterial targets [33]. Shortly after their discovery, phages were recognized for their potential to combat bacterial infections, with the first generation of phage-based treatments relying on the natural specificity and lytic action of wild-type viruses [32,34,35]. Though their specificity presents an advantage at face-value, a narrow targeting spectrum without equally specific diagnostics for identifying the infectious pathogen, as well as the selection of resistance to phages during phage-based treatments, fueled the development of therapeutic cocktails that consisted of multiple phage species, each with different targeting spectrums. However, with the discovery of penicillin and other potent antibiotics, efforts to develop phage-based therapies were largely abandoned.

As the efficacy of antibiotics threatens to continue its decline in the face of growing resistance, interest in developing phage-based therapeutics has regained traction. Genetic manipulation of phages

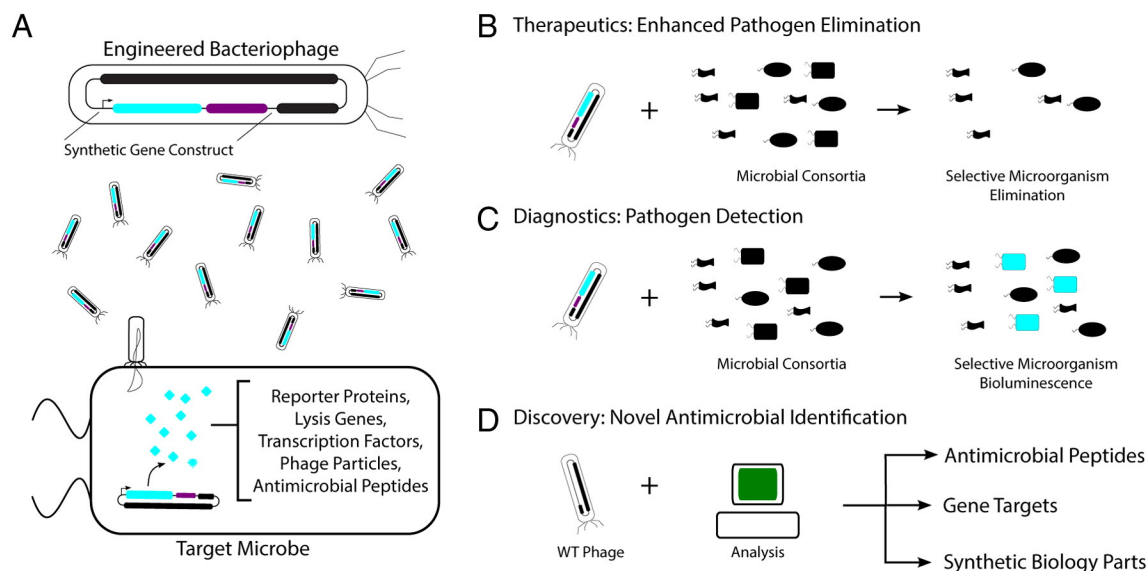


Fig. 1. Engineered phages. (A) The evolved ability of phage to infect and replicate within a host microorganism has made them a popular technology platform. An engineered phage can induce the expression of heterologous constructs in a specific population of microbes for therapeutic and diagnostic applications. (B) Modifying the phage genome to include the expression of bactericidal genes enables the utilization of phage as an antimicrobial therapeutic for specific microbial elimination. (C) By inducing microbial expression of reporter proteins, an engineered phage can facilitate bioluminescence that enables precise and sensitive detection of a microorganism. (D) Mining phage biology can aid in the identification of novel antimicrobial peptides, essential gene targets, and synthetic biology parts.

has propelled their use as a valuable platform for targeted bacterial elimination (Fig. 1B). Recent work has aimed to widen phage target range by introducing genes that allow for replication in new bacterial hosts and by re-engineering phage tail fibers to enable the infection of new targets [36–43]. Recently, Ando *et al.* described a high-throughput method for engineering novel phage specificity in a modular manner. These synthetic phages can be inexpensively and rapidly evaluated for clinical use, easing the path for regulatory approval and advancing the therapeutic potential of phage-based treatments [34,44–46].

Genetic modifications have also been used to bolster phage antibacterial activity. For example, Lu *et al.* modified T7 phages to express a biofilm-degrading enzyme, Dispersin B (DspB) [47]. With DspB's activity, each successive round of phage replication led to biofilm disruption and diffusion of phage progeny through the biofilm matrix to promote infection clearance. However, cytotoxic cellular components released during bacterial lysis can lead to inflammation and deleterious immune responses *in vivo*, motivating the exploration of alternative phage-based methods for inducing microbial death [32,33,41,48,49]. Second-generation phage therapeutics rely on non-lytic (lysogenic) phage and non-replicating phage protein capsules (phagemids) to deliver gene networks programmed to eliminate bacterial populations through non-lytic means. Phage-delivered antibiotic-sensitizing factors designed to render bacterial populations hyper-susceptible to standard treatments have been developed as adjuvant therapies. For example, lysogenic phages have been engineered to overexpress transcription factors associated with repression of SOS response and DNA repair, leading to increased antibiotic susceptibility in infected cells [50]. Lysogenic phages have also been modified to reverse antibiotic resistance by inducing microbial expression of dominant-sensitive genes that lead to antibiotic re-sensitization [51]. Additionally, phagemid-induced expression of small regulatory RNAs has been used to knock down the expression of resistance genes in order to recover antibiotic susceptibility [52].

Phage-delivered synthetic networks have also been utilized to directly kill bacteria without antibiotic co-treatment. In a recent example, Krom *et al.* engineered phagemids to induce the expression of non-lytic antimicrobial peptides (AMPs) and toxin proteins, leading to targeted non-lytic bacterial death [53]. This system is modular, allowing for rapid plasmid construction, AMP screening, and AMP expression tuning. The platform's extensible design for testing new AMPs and its demonstrated efficacy *in vivo* highlight recent advancements in phage-based therapeutics and their potential for clinical applications [53,54].

There have also been advances in harnessing the CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated genes) system for antimicrobial therapeutics [55]. Originally identified as a component of the prokaryotic adaptive immune system whereby the endonucleolytic Cas9 protein is guided to cleave foreign DNA species by a guide RNA complex, CRISPR/Cas was soon recognized for its potential to better enable targeted recombination of DNA [56–61]. Since then, the CRISPR/Cas system has been broadly adopted as a genome editing tool in a variety of host organisms [23,55,62–69]. CRISPR/Cas has also been used to eliminate select bacterial strains in mixed populations, but delivery challenges have stymied the system's development as a therapeutic agent [55,70]. These delivery constraints were recently addressed by the exploitation of non-lytic phagemid-mediated delivery. This approach utilized phagemid specificity to selectively deliver a CRISPR-guided nuclease programmed to cleave antibiotic resistance genes. Directed nuclease action prevented plasmid-borne resistance in antibiotic-sensitive populations, while simultaneously eliminating antibiotic-resistant microbes [71,72]. The efficacy of phage-mediated CRISPR/Cas delivery has been demonstrated in both Gram-positive and Gram-negative organisms, as well as in *in vivo* infection models, emphasizing the widespread applicability of this platform technology.

Although phagemid-delivered CRISPR/Cas systems boast high specificity and multiplexed targeting capabilities, there is concern regarding the potential emergence of phage-resistant microbial populations as a

result of phage treatment. Yosef *et al.* addressed this hurdle by using lytic phage and CRISPR/Cas to induce a population-wide selective advantage for antibiotic-sensitive microbes [73]. Temperate phages were used to deliver a CRISPR/Cas construct that both eliminated microbial plasmids that bestowed antibiotic resistance and immunized target bacteria against lytic phage infection. This tactic left CRISPR/Cas-treated bacteria sensitive to antibiotics while simultaneously conferring an evolutionary advantage against lytic phage attack. Thus, antibiotic-resistant cells could be efficiently eliminated by lytic phages while antibiotic-sensitive populations were left to take over the microbial niche, paving the way for efficacy of standard antibiotic therapies.

2.2. Synthetic probiotics as living therapeutics

The ability to engineer bacteria has enabled antimicrobial therapies in the form of synthetic probiotics that can provide localized and controlled release of targeted therapeutics under specific conditions, such as the onset of an infection (Fig. 2A). Live attenuated pathogens have long been utilized as vaccines, stimulating the host immune system to produce relevant antibodies for long-term prophylaxis [74]. Improvements in genetic engineering methods have led to the creation of recombinant bacteria for treatment and prevention of infectious disease [75–77]. Early efforts consisted of engineering benign bacteria to express heterologous antigens and evoke a host immune response (Fig. 2B) [78–83]. In later iterations, non-pathogenic microbes were modified to display toxin receptor-mimics that act as competitive inhibitors of pathogenic toxins [84–86]. Other approaches used engineered probiotics to disrupt pathogen quorum sensing and prevent virulent gene expression, demonstrating a generalizable method of interfering with pathogen communication for antimicrobial purposes [87,88]. Additional advancements in probiotic-based therapeutics focused on promoting long-term viability of recombinant bacteria by engineering commensal species that would be expected to stably colonize the mammalian host [87]. Prophylactics for human immunodeficiency virus (HIV) were developed using this approach, by modifying *Lactobacillus* and *Escherichia coli* (*E. coli*) species that naturally colonize the cervicovaginal tract to secrete antiviral peptides [89–91].

Synthetic probiotics can also be engineered to colonize particular niches by heterologous expression of localizing peptides. Such probiotics convey prophylactic effects by outcompeting pathogens for ecological niches and by employing conventional strategies for toxin inhibition [92,93]. Targeted therapeutic delivery has also been achieved by engineering an attenuated version of an otherwise pathogenic strain. For example, the natural macrophage-homing mechanism of *Salmonella* has been used to introduce antigenic peptides to immune cells for prophylactic purposes, and for the delivery of ribozymes designed to inhibit macrophage-specific viral infections, such as cytomegalovirus [94–97]. The employment of attenuated *Salmonella* in vaccine delivery has promising commercialization potential and is currently the basis of several clinical trials [98,99].

There is also interest in developing autonomous probiotics that are able to sense particular pathogens and respond by expressing targeted antitoxins or antimicrobial agents (Fig. 2C). Saeidi *et al.* engineered *E. coli* to sense *Pseudomonas aeruginosa*'s quorum sensing molecule and respond with self-lysis to prompt the release of a narrow-spectrum antimicrobial agent [100]. Similarly, *Lactococcus lactis* was designed to respond to an *Enterococcus faecalis* sex pheromone with the secretion of a suite of AMPs [101]. These efforts provide platforms for the future development of synthetic probiotics that provide long-term prophylactic and therapeutic benefits.

However, challenges remain in translating engineered probiotics to clinical applications. Stable long-term colonization of a therapeutic probiotic in a microbial niche has proven difficult to achieve with standard laboratory strains, motivating the domestication and study of commensal microorganisms that can be used for this purpose [102]. Additionally, engineering the robust function of synthetic gene networks is challenging

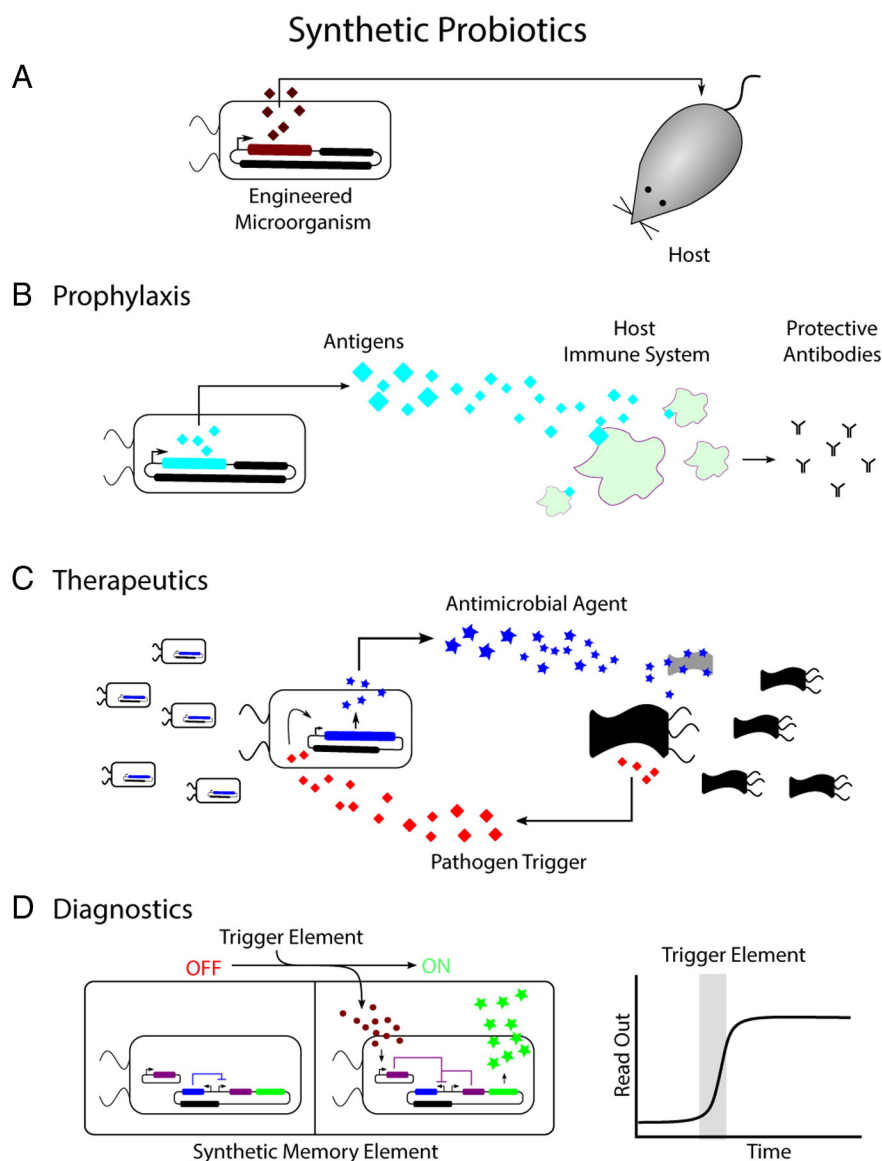


Fig. 2. Synthetic probiotics. (A) Engineered microorganisms can be deployed *in vivo* to provide prophylactic, therapeutic, and diagnostic benefits. (B) By engineering the secretion of antigenic factors, a non-pathogenic microbe can be used to safely induce a protective host immune response to a particular pathogen. (C) Autonomous therapeutic probiotics that sense and respond to the presence of a pathogen have enabled the targeted killing of infectious microbes. (D) By modifying probiotic microbes to express synthetic gene circuits that facilitate cellular memory, probiotics can be used to persistently monitor the host and report on the presence of a molecule or signal of interest.

in the face of *in vivo* environmental pressures. Efforts to create and study synthetic microconsortia may be useful in gaining perspective on dynamic *in vivo* environments and advances in gene network engineering may facilitate the robust function of therapeutic circuitry [103,104].

Another concern associated with the use of living, genetically engineered microorganisms is their potential for proliferation in natural environments [105]. Early approaches to biological containment involved engineering auxotrophy via essential gene knockouts which render the host incapable of replication in the absence of essential metabolites, and inducing microbial death by controlled expression of lethal genes [106–109]. Building on these efforts, Chan *et al.* developed genetic Deadman and Passcode networks that allow for tightly controlled and programmable cellular killing [110]. The Deadman circuit utilizes unbalanced reciprocal repression networks to control expression of a toxin protein that induces host death only in the absence of an exogenous ligand. By coupling this circuit with a network of hybrid transcription factors, the authors were able to induce microbial death in response to the simultaneous presence of up to four exogenous ligands. These safeguard systems efficiently kill engineered microbes

upon either exposure to a small molecule or expulsion of the microbes from the patient's body.

3. Diagnostics

The societal and monetary costs associated with the lack of effective diagnostic measures calls for improved antimicrobial testing capabilities [2,3]. Clinically tractable diagnostics must be low-cost, rapid, sensitive, easy to use, and adaptable to new targets. With their rational design, synthetic biology platform technologies hold promise for diagnostic technologies that can address these needs.

3.1. Bacteriophage-based diagnostics

Phages are ideal vectors for diagnostic applications due to their highly specific targeting capabilities [111,112]. Early phage diagnostics took the form of plaque-assays that identified bacteria based on their clearance by known phage species [34,111–113]. With time, phage-based diagnostics evolved to rely on genetic modifications to enhance

signal amplification and shorten the time required to obtain a readable output [33]. Phage-infection markers have taken the form of standard fluorescent, bioluminescent, and colorimetric readouts (Fig. 1C) [112–117]. In other approaches, external phage proteins have been genetically modified to allow for detection through auxiliary methods. In a demonstration of this methodology, Edgar *et al.* engineered phages to express a biotinylated capsid protein for direct detection by streptavidin-coated quantum dots, allowing identification of as few as ten bacterial cells per milliliter in one hour [118]. While modern-day clinical use of phage-based therapeutics has been dampened by strict regulatory requirements, commercial diagnostic applications of phages have had considerable success, with several such platforms available for use in laboratory and food-industry settings [33,34,111,119].

3.2. Cell-free and paper-based platforms

Although phages are suitable for a wide range of extracellular diagnostics, other biologically-based diagnostic platforms are less tractable *in vitro*. Progress in synthetic biology has led to improved circuit-building abilities and a large collection of RNA and DNA sensors; however, these technologies fundamentally require transcription and translation, limiting their applicability to cellular contexts [21,22,120,121]. *In vitro* cell free expression systems that contain transcription and translation machinery provide the environment necessary for biologically-based technologies to function independently of living cells [122]. Originally utilized for *in vitro* protein expression, cell free expression systems have recently been recognized for their ability to support more complicated gene networks [123–126]. Synthetic gene circuits have been shown to operate well within these systems, often with the added benefit of reduced cross-talk and minimal off-target effects. Further, the more precise control of conditions offered by *in vitro* expression systems has enabled the high-throughput tuning of synthetic constructs [123,124,127,128]. Despite these advancements, the application of cell-free methods has been limited to laboratory settings due to strict storage requirements, including the need for refrigeration.

To mitigate these constraints, Pardee *et al.* recently developed a paper-based system for cell-free gene expression, providing a simple platform for the real-world application of engineered genetic systems. (Fig. 3) [129]. This paper-based technology utilizes cell-free extracts freeze-dried on paper and other porous substrates to allow for long-term preservation of synthetic circuits at room temperature. In a noteworthy demonstration, RNA toehold switch sensors were used to develop a paper-based colorimetric diagnostic that was able to rapidly detect and distinguish between the Sudan and Zaire Ebola strains from the 2014 outbreak [22,129,130]. The paper-based system has also been shown to integrate well with non-RNA sensor modalities such as ligand-inducible promoters and FRET-based nanosensors, and to allow for complex circuitry that encodes molecular-based logic. This platform is primed for antimicrobial diagnostic development and represents a scalable, cost-effective technology that is sensitive, easy to use, and compatible with synthetic biology tools.

3.3. Synthetic probiotics as living diagnostics

Although most diagnostics have been developed for *in vitro* use, there has been a recent push toward the development of *in vivo* living diagnostic platforms. Much like therapeutic probiotics that secrete antimicrobial agents upon pathogen detection, diagnostic bacteria could persistently monitor the microbiome for a particular cue and respond with reporter expression. One key difference between therapeutic and diagnostic probiotics is the fundamental need for the latter to encode long-term synthetic memory. A memory device ensures that the diagnostic microbe will “remember” transient environmental events even after the trigger has disappeared and will convey accurate diagnostic information over the course of multiple cellular generations (Fig. 2D). Early efforts to engineer genetic memory focused on the creation of bistable toggle switches that flipped between two protein expression states in response to exogenous inducers [7] and native cellular networks such as the SOS signaling pathway responding to DNA damage [131]. More recently, efforts to engineer biological memory transitioned to the use of DNA modification in order to overcome

Paper-Based Diagnostics

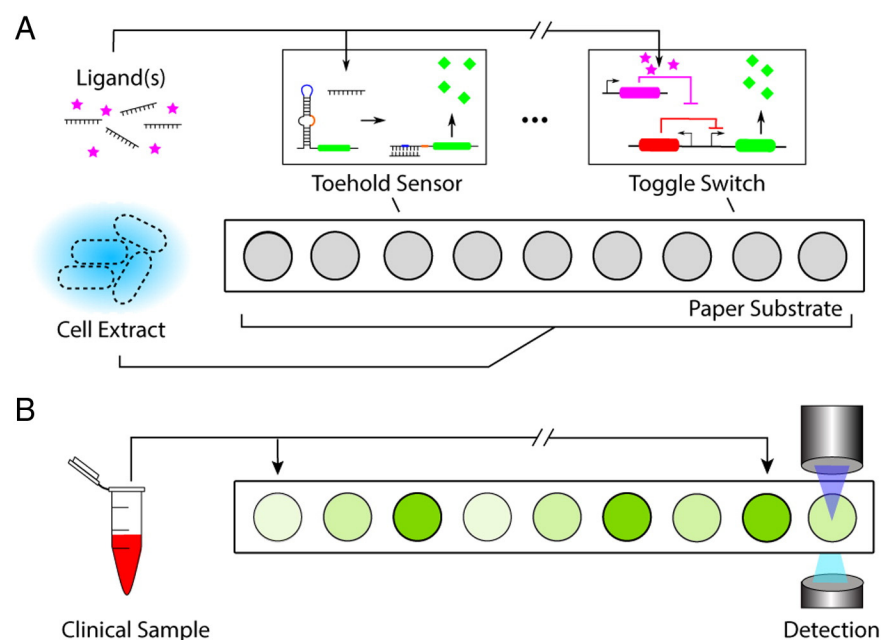


Fig. 3. Paper-based diagnostics. Paper-based diagnostics enable the widespread use of synthetic gene constructs in clinical applications. (A) Freeze-dried cell extracts containing a synthetic gene network designed to detect clinically relevant signals are spotted on a paper substrate. Subsequent addition of a clinical sample triggers a colorimetric change that can be assayed for diagnostic relevance. (B) Freeze-dried expression systems facilitate the operation of genetic systems *ex vivo*, enabling the use of synthetic gene platforms for precise diagnostics in a low-cost and tractable manner.

inherent stochasticity in gene expression and relieve the metabolic load imposed on the host microbe in sustaining circuit operation. Orthogonal integrases that can irreversibly flip DNA segments have been engineered to encode genetic memory [132,133], and recombinase-based systems have been used to record the magnitude and duration of trigger exposure through regulated co-expression of recombinase and retron elements that modify genomic DNA [134].

Improvements in the design of synthetic cellular memory have led to the prospect of building reliable diagnostic probiotics for use *in vivo*. Researchers have endowed *E. coli* with a synthetic memory circuit that enables probiotic tracking of antibiotic exposure from within a mouse gut [135], and the human commensal microbe, *Bacteroides thetaiotamicron*, has been instilled with integrase-based memory constructs that can operate *in vivo* to track external stimuli [102]. These efforts demonstrate the budding feasibility of using engineered probiotics to persistently monitor the gut microbiome.

Thus far, most synthetic probiotics have been proof-of-concept models designed to respond to exogenous inducers rather than to host factors indicative of a diseased state or pathogen presence [136]. Mining natural bacterial pathways can help develop more relevant sensing capabilities, such as the detection of nitric oxide levels that are indicative of gut inflammation [137]. To further expand the repertoire of pathogen sensors, development of chimeric transcription factors that connect a desired signal to a known transcriptional output may be a productive strategy [138]. Such efforts have been successful in engineering novel ligand-inducible expression systems that respond to factors such as amino acids and light [139,140]. Expanding the ability of engineered probiotics to sense their environment will greatly improve their clinical relevance as diagnostic and therapeutic agents.

4. Discovery

In the pursuit of antimicrobial therapeutics and diagnostics, effort must also be invested in identifying novel antimicrobial targets and agents. Several synthetic biology platforms offer opportunities to progress these goals by advancing technologies for the continued study of virulent microorganisms.

4.1. Bacteriophage-based discovery tools

Although the advancement of phage-based therapeutics largely stalled with the introduction of antibiotics, phages have long been used as experimental platforms in many aspects of microbiological research (Fig. 1D). Phage-based library screens, genome engineering techniques, and accelerated evolution platforms have been utilized for microbial research and therapeutic development, and many synthetic biology devices have been derived from natural phage biology [141]. These methods are increasingly common in research labs and have been employed extensively to advance our understanding of infectious microbes. In a unique application of phage genomics, Liu *et al.* described a protocol for mining a phage component library for novel antimicrobial agents and therapeutic targets [142]. Demonstrated in *Staphylococcus aureus*, this generalizable protocol can be employed in the study of other phage/bacterial species pairs.

4.2. CRISPR/Cas for microbial screening and genome editing

As described in Section 2.1, CRISPR/Cas has become a ubiquitous genome-editing tool within biological research. The CRISPR/Cas system can be used to study the pathogenicity of clinically relevant intractable pathogens due to its portability to a wide variety of organisms. In a recent demonstration, Ghorbal *et al.* described the use of CRISPR/Cas in the malaria parasite, *Plasmodium falciparum* [143]. By introducing specific gene knockouts and single nucleotide substitutions, the group was able to engineer an artemisinin-resistant *Plasmodium* strain to validate the resistance causality of a previously implicated genetic

polymorphism. CRISPR/Cas has also enabled the generation of luciferase-reporter *Cryptosporidium* parasites, opening the door to rapid drug screening for this diarrheal pathogen that is one of the leading causes of global child mortality [144]. CRISPR/Cas systems have additionally been applied as high-throughput screening tools, employing guide RNA libraries for wide-scale targeted mutagenesis and gene knockout library generation in mammalian and yeast cell lines, holding promise in pathogenic applications [145–148].

4.3. Synthetic microbial-screening platforms

Though initially developed for cellular engineering pursuits, several devices used in synthetic biology have exhibited functionality as effective screening platforms and discovery tools. Riboregulators are RNA-based devices that confer tunable control of gene expression via post-transcriptional regulation [21,150,151]. These mediators have been used in conjunction with systems-level transcriptional profiling to study microbial responses to the induced imbalance of a toxin-antitoxin system [150], as well as to gain insight into the underlying mechanisms of action of antibiotic treatments [152,153]. Green *et al.* recently developed a second-generation riboregulator variant that enables tunable regulation of endogenous RNA transcripts and can be employed in additional microbial screens [22]. Another synthetic biology platform that can be used for antimicrobial screening and target evaluation is a recently developed tagged protein degradation system [154]. Cameron *et al.* engineered inducible, selective protein degradation in *E. coli* and *L. lactis* through expression of an orthogonal protease system. This platform has demonstrated utility in characterizing putative antibiotic adjuvant targets. For example, the induced degradation of RecA (an endogenous DNA repair protein) led to a hypersensitive phenotype in co-treatment with norfloxacin (a DNA-damage-inducing antibiotic), confirming that a RecA inhibitor could function as a viable norfloxacin adjuvant. Furthermore, the researchers generated an Essential Protein Degradation (EPD) library in *E. coli* consisting of 238 degradation-tagged essential genes to promote broad use of this platform in identifying new antimicrobial and adjuvant moieties.

4.4. Engineered microbial communities

Medical and scientific communities are increasingly mindful that antibiotic resistance can be transferred between and among species coexisting in a single population, and that broad-spectrum antibiotics have negative consequences on the native microbiome [30,155]. However, the mechanisms underlying these processes are not entirely understood. To this end, synthetic biologists have begun to engineer microbial ecosystems that contribute to our understanding of microbiome ecology and may lead to new therapeutic and vaccine strategies [30, 103,104,156]. Construction of synthetic predator-prey ecosystems in which “predator” microbes were modified to induce and survive off of the lysis of “prey” bacterial strains suggests that even simply-defined inter-species relationships can lead to complex population dynamics [157]. The emergence and stability of cooperative syntrophic exchange have also been investigated by knocking out essential amino-acid biosynthesis genes and analyzing the resulting population dynamics in engineered co-dependent microbial consortia [158]. In alternate schemes, CRISPR/Cas therapeutic technologies have been repurposed to study the effects of selective manipulation of heterologous microbial populations [71,72]. These targeted methodologies can be used to study interactions between species *in vitro* and can also be employed to potentiate *in vivo* simulations of microbial niche clearing, a phenomenon known to lead to acutely intractable infections such as *C. difficile* [4]. Additionally, sentinel prokaryotic systems may be engineered to monitor environmental stimuli *in vivo* to investigate native interactions within the microbiome [134,135].

5. Conclusions

Advancing the next generation of antimicrobial therapies represents a unique challenge and opportunity for the practical application of synthetic biology tools. The microbial landscape is constantly changing as bacteria evolve and respond to the treatments that are developed to combat them. Synthetic biology rationale can aid in the rapid development and tuning of new therapies that are required to keep pace with these evolving adversaries. On the diagnostic front, new paper-based technologies provide the means for low-cost, rapid testing in clinical and/or resource-limited settings, as the first step in determining proper antimicrobial treatment. Additional synthetic biology platforms, such as phage-based technologies and attenuated *Salmonella*-based vaccines, currently exist as commercially viable entities, while others, such as synthetic probiotics, are rapidly improving and will soon join the ranks in real-world deployment. Synthetic biology platform technologies are well poised to contribute to the development of novel therapeutic, diagnostic, and discovery modalities in a wide range of antimicrobial applications.

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